IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ORIGINAL

In re application of:

ZAUDERER *et al.*

Appl. No. 09/987,456

Filed: November 14, 2001

For: ***In Vitro* Methods of Producing and Identifying Immunoglobulin Molecules in Eukaryotic Cells**

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Declaration Under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, Dr. Walter J. Storkus, residing at 3303 Mount Royal Boulevard, Glenshaw, PA 15116, USA, declare and state as follows:

1. I was a member of the Scientific Advisory Board ("SAB") of Vaccinex, Inc., from 2001 until 2004.

2. A current *curriculum vitae* is appended hereto as Exhibit A1.

3. I received my Ph.D. degree in Microbiology and Immunology from Duke University in 1986, evaluating the importance of MHC class I molecule expression by tumor cells in their ability to be recognized and killed by natural killer (NK) cells. Since that time, I have been an NIH-funded investigator targeting the development of tumor vaccines and immunotherapies for patients with cancer. I am currently a tenured full professor in the

Departments of Dermatology and Immunology at the University of Pittsburgh. As seen from my attached *curriculum vitae*, I have published extensively, received many honors, and am involved in numerous professional and scientific societies related to immunology. Based on my education and experience, I am an expert in the field of immunology.

4. I have reviewed the above-identified patent application ("patent application"), the final Office Action dated April 21, 2005; PCT Publication No. WO 93/01296 to Rowlands *et al.* ("Rowlands"); PCT Publication No. WO 93/01296 to Zauderer ("Zauderer"); and Waterhouse *et al.*, *Nucleic Acids Res. 21*:2265-2266 (1993) ("Waterhouse") (collectively, "the cited references"). I have also reviewed the pending claims of the patent application.

5. The invention claimed in the patent application relates to the field of immunology. More particularly, the invention relates to the art or field of methods of identifying, producing, and/or expressing immunoglobulins in eukaryotic cells.

6. When Dr. Maurice Zauderer, a co-inventor of the captioned application and President and CEO of Vaccinex, Inc. ("Vaccinex") first presented the idea of the present invention to the SAB of Vaccinex, Inc., the SAB members and I were skeptical that the present invention would succeed. The reasons for my skepticism are explained in detail below.

7. The natural human immune system is necessarily an incredibly complex one that requires vast numbers of B cells interacting with antigen presenting cells in highly organized tissues in order to achieve the diversity necessary to produce antigen specific antibodies. At the time the idea for the present invention was presented to me, I did not think that antigen-specific antibodies could be efficiently selected from random libraries of immunoglobulin heavy and light chains expressed in eukaryotic cells *in vitro* because I thought specific antibodies of interest would occur at relatively low frequency and it would not be practical to screen the number of eukaryotic cells necessary in order to find an antibody that had specificity for a specific antigen of interest. I and the other members of the SAB were aware of reports that this could be done for libraries of antibody fragments expressed in phage. This did not, however, convince us that good antibodies could be selected in eukaryotic cells because: 1) the throughput for screening phage exceeded the expected throughput for screening libraries expressed in eukaryotic cells by as much as four orders of magnitude; 2) most of the work reported with antibody fragments expressed in phage was carried out with single chain Fv (scFv) in which the variable regions of immunoglobulin heavy and light chains are covalently linked, thereby increasing the likelihood that they will associate. This is significant because one of the concerns raised in the SAB was that antibodies are efficiently assembled and expressed in mature B lymphocytes because their component immunoglobulin heavy and light chains have been selected to pair properly. In contrast, we thought that random pairs of immunoglobulin heavy and light chains derived from separate libraries would be poorly matched and would, therefore, fail to associate properly in the eukaryotic cytoplasm. A related point is that antibody fragments expressed in phage, whether or not the immunoglobulin heavy and light

chain variable regions are covalently linked, concentrate and are assembled in the periplasmic space. The conditions of assembly in the eukaryotic cytoplasm are far different from those that apply in the periplasmic space and it could not be known what effect this would have on antibody assembly. This difference in the conditions of assembly may explain some of the many examples of antibodies selected from phage libraries that cannot be expressed in eukaryotic cells. Finally, 3) based on a biopharmaceutical industry survey commissioned by Vaccinex and carried out by L.E.K. Consulting (28 State St., Boston, MA 02109) in the Fall of 2001, "*Monoclonal Antibody Partnerships in the Biopharmaceutical Industry*" (attached hereto as Exhibit A2), we understood that the quality of antibodies selected from phage libraries was, at that time, viewed by senior industry executives as a research tool that, in comparison to immunoglobulin transgenic mice or humanization of murine antibodies, was not good enough or consistent enough for commercial therapeutic applications. Although Vaccinex management cited this as evidence of an unmet need for a library-based technology, it also highlighted the difficulty of the challenge. There were, at the time the present application was filed, no grounds for confidence that antibodies selected from libraries expressed in eukaryotic cells would be any more suitable for commercial therapeutic use than those selected from libraries expressed in phage.

8. Since the time the Vaccinex patent application was filed, there have been significant improvements to phage display technology and, especially, to the quality and diversity of immunoglobulin variable gene libraries expressed in phage. This has had a beneficial impact on the quality of antibodies selected using phage technology. Similarly, in spite of our concerns, selection of high quality antibodies from immunoglobulin gene libraries expressed in eukaryotic cells, as carried out at Vaccinex after the present

application was filed, has also proven quite successful. Apparently, random pairs of immunoglobulin heavy and light chains often do associate successfully and the efficiency of antibody assembly in eukaryotic cells is so much higher than in bacteria that antigen-specific antibodies can still be selected even though the throughput for screening libraries expressed in eukaryotic cells is much lower than for libraries expressed in phage. The key point is that, at the time the present application was filed, we could not have known that this technology would be successful.

9. The success of this invention would not have been expected based on the Rowlands, Zauderer and Waterhouse references. My expectations would not have changed in view of Rowlands, because Rowlands only demonstrated expression of a single antibody which had already been selected for immunoglobulin heavy and light chains that paired correctly and efficiently. This is a far simpler problem than expression of a large number of random pairs from two separate libraries. My expectations would also not have changed in view of Zauderer, because only one library was introduced into host cells. Therefore, Zauderer did not address the concern of assembling separate chains derived from two random libraries as in the present invention. Furthermore, although Waterhouse does suggest introducing a library of heavy chain fragments and a library of light chains into *E. coli* host cells for phage display of antibody fragments, I would not have expected that two random libraries could be introduced into eukaryotic host cells to provide antigen-specific antibodies. First, and most importantly, the Waterhouse technique was developed as an improvement for phage display, which is a system using prokaryotic host cells. As such, the Waterhouse technique could not simply be extrapolated into a eukaryotic host cell system. Second, in the specific examples of Waterhouse, it was not shown that using two libraries to

select a previously unidentified antigen-specific antibody would actually work in a prokaryotic phage display because the data described therein only shows the results of using a few immunoglobulin sequences that had previously been identified. I am aware of separate work by others (*e.g.*, H.J. de Haard *et al., J Biol Chem*, 274:18218-18230, 1999, attached hereto as Exhibit A3) in which a library of heavy chain fragments and a library of light chains were introduced into *E. coli* host cells for phage display of antibody fragments, but, again, it would not have been possible to extrapolate from the conditions of assembly of antibody fragments in the periplasm of bacteria to assembly of immunoglobulin molecules in a eukaryotic system.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Walter J. Storkus, Ph.D.

Date: 7/20/05